PATENT APPLICATION
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	I hereby certify that this paper is being deposited with the United States Postal Service as first class mail, postage prepaid, in an envelope addressed to the Assistant Commissioner for Patents, Washington, DC 20231, on this date:
Masinovsky et al.	
Serial No: 08/448,649	
Filed: May 24, 1995	
For: METHODS FOR USING AGENTS THAT BIND TO VCAM-1 (Amended Title)	Date: _____
Group Art Unit: 1815	_____ Li-Hsien Rin-Laures, M.D. Reg. No. 33,547 Attorney for Applicants
Examiner: P. Gambel, Ph.D.	

DECLARATION OF THALIA PAPAYANNOPOULOU, M.D., Dr. Sci., UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

I, Thalia Papayannopoulou, M.D., Dr. Sci., hereby declare and say as follows:

1. I received my M.D. and my Dr. Sci. from the University of Athens in 1961 and 1964, respectively. I completed my internship and Assistantship in Medicine and Hematology in Greece. I was an NIH Fellow and Senior Fellow in the Department of Hematology at the University of Washington, Seattle, Washington. Since 1978, I have been on the faculty of the Department of Medicine of the University of Washington. I have been a Professor in the Division of Hematology since 1984 and Director of the Hematology Training Program since 1990. I have been on numerous committees of the American Society of Hematology and the International Society of Experimental Hematology. I was awarded the William Dameshek Prize by the American Society of Hematology in 1990. I have authored or

co-authored at least 199 publications. I have done extensive research into hematopoietic stem cells, including peripheralization of hematopoietic stem cells.

2. Experiments were performed at my direction to assess the ability of antibody 6G10 to cause *in vivo* release of bone marrow progenitor cells from the bone marrow of animals following systemic administration of antibody 6G10 to the animal. As described in more detail below, antibody 6G10 was administered to two non-human primates and levels of bone marrow progenitor cells in bone marrow aspiration samples and peripheral blood samples were assessed before, during and after antibody 6G10 administration. The resulting data is set forth in Tables 1 and 2 below. The day of the first injection of each animal is denoted as day "0".

3. In one experiment, the test animal was a *Macaque nemestrina* monkey. Antibody 6G10 was administered intravenously to the animal on three consecutive days (days 0, 1 and 2) at a dose of 2.0 mg/kg. Peripheral blood samples were taken prior to the first injection and at 1, 2, 3, 4 and 7 days following the first injection. Bone marrow aspiration samples were taken prior to the first injection and at 4 days following the first injection. Samples were collected in preservative-free heparin.

4. Mononuclear cells from the peripheral blood and bone marrow samples were isolated by centrifugation on a cushion of Lymphoprep. Interface cells were washed and cultured in methylcellulose medium consisting of 0.9% methylcellulose, 50% bovine serum, 1% bovine serum albumin, and 0.1 mM 2-mercaptoethanol in Iscove's modified Dulbecco's medium. Also present in the culture medium were erythropoietin (2 units/ml), Kit ligand/stem-cell factor (50 ng/ml), granulocyte/macrophage-colony-stimulating factor (50 ng/ml), and gibbon interleukin-

3 (50 units/ml). All cultures were set up in duplicate or triplicate plates with cells plated at three cell concentrations between $1x10^5$ to $1x10^6$ cells/ml. The cells were incubated at 37°C with 5% CO_2 at high humidity. Erythroid burst-forming units (BFUe) and granulocyte/macrophage-colony-forming units (GM-CFU) were counted in plates of live cells 12-14 days after plating on the basis of morphological criteria observed with a dissecting microscope.

5. Data from the experiment described above in paragraphs 3 and 4 are shown in Table 1 below. Prior to the first injection of antibody 6G10, in the peripheral blood samples there were 12.4 BFUe/ml blood and 74.5 GM-CFU/ml blood. After administration of antibody 6G10 began, in the peripheral blood the concentration of BFUe rose as high as 30.8 BFUe/ml blood on day one and the concentration of GM-CFU rose as high as 324.3 GM-CFU/ml blood on day two.

TABLE 1

Day	PERIPHERAL BLOOD		BONE MARROW	
	BFUe/ml	CFU/ml	BFUe/ml	CFU/ml
0	12.4	74.5	$1.27x10^5$	$1.56x10^5$
1	30.8	288		
2	9.7	324.3		
3	0	106.7		
4	0	106.4	$3.51x10^5$	$8.15x10^5$
7	0	78.1		

6. In another experiment, a *Macaque fascicularis* monkey was treated with one intravenous injection of antibody 6G10 at a dose of 2.0 mg/kg. Peripheral blood samples were taken prior to the injection and at 1, 2, 3, 4 and 7 days following the injection. Bone marrow samples were taken prior to and at 2 days following the injection. The samples were obtained, cultured and analyzed as described above except red cells in the peripheral blood samples were lysed with hemolytic buffer.

7. Data from the experiment described above in paragraph 6 are shown in Table 2 below. Prior to the injection of antibody 6G10, in peripheral blood samples there were 19.8 BFUe/ml blood and 118.8 GM-CFU/ml blood. After administration of antibody 6G10, in the peripheral blood the concentration of BFUe rose as high as 76.9 BFUe/ml blood seven days following the injection and the concentration of GM-CFU rose as high as 351.9 GM-CFU/ml blood four days following the injection.

TABLE 2

Day	PERIPHERAL BLOOD		BONE MARROW	
	BFUe/ml	CFU/ml	BFUe/ml	CFU/ml
0	19.8	118.8	$2.75x10^4$	$5.6x10^4$
1	39.15	102.2		
2	38.6	101.6	$2.05x10^5$	$1.73x10^5$
3	7.5	224.3		
4	22.2	351.9		
7	76.9	333.8		

8. The above data show that antibody 6G10 causes release of bone marrow progenitor cells from the bone marrow to the peripheral blood following systemic administration of antibody 6G10 to non-human primates.

9. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

11-30-1995
Date

Thalia Papayannopoulou, M.D., Dr. Sci.